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ASSAY FOR AFLATOXINS IN SOME LOCAL FOOD CONDIMENTS

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ABSTRACT

Aflatoxins are secondary metabolites with toxicological properties that induce a variety of health challenges when foods contaminated with these compounds are ingested. Aflatoxins are stable under most food processing conditions and therefore persist to the final products. Fresh samples of thirty two food condiments comprising of four each of *Pleurotus tuber regium* (Osu), *Piper guineense* (Uziza), *Xylopia aethiopica* (Uda), *Monodora mystrica* (Ehuru), *Citrullus vulgaris (*fermented mellon), *Ricinus communis* (Ogiri) *Brachystegia eurycoma* (Achi) and *Origanum syriacum* (Offor) were assayed for fungi and presence of aflatoxins. The mean fungal counts range from 1.2 x 10^4 in *Pleurotus tuber regium* to 8.2 x 10^9 in *Brachystegia eurycoma*. Eight fungal spp were identified to include Aspergillus, Penicillium, Candida, Mucor, Rhizopus, Geotricum, Saccharomyces and Fusarium. Aflatoxin assay by RIDA quick aflatoxin kit detected two samples of *Brachystegia eurycoma* and a sample of *Origanum syriacum* with concentrations ≥ 20ppb. The best protection against mycotoxins is monitoring their presence in foods and feeds, therefore, regular and routine analysis of food samples for possible presence of mycotoxins is recommended. Education and training of food handlers is imperative, adoption of GMP and HACCP application in food condiment preparation will curtail fungal contamination and mycotoxin production in foods and feed products.

Keywords: Food Condiments, Aflatoxins, Mycotoxins, Food Handlers, GMP, HACCP

INTRODUCTION

Food condiments (seasonings/spices) are substances added to food to impart a particular flavor, enhance its flavor or in some cultures to complement the dish. Most locally

made food condiments are fermented and their production still shrouded in the local act of those who inherited it as a family secrete/trade gift by nature. More often, production by local technology creates room for contamination by diverse microorganisms, and the fermentation environment support and encourages fungi growth and proliferation and thus mycotoxin production.

Mycotoxins are diverse range of molecules, they are secondary metabolites produced by fungi which contaminate a large variety of food and feed. Fungi are extremely adaptable organism, being able to metabolize a large variety of substrates over a wide range of environmental conditions, thus no region of the world escapes the problem of mycotoxins and according to Lawlor and Lynch, 2005, **[1]**, mycotoxins are established to affect as much as 25percent of the world's crops every year.

Plants may be contaminated by mycotoxins in two ways; fungi as pathogens on plants or growing saprophytically on stored plants and animal products **[2]**. Most mycotoxins in food are produced mainly by three genera of fungi; Aspergillus, Penicillium and Fusarium **[3]**.

Due to the diversity of their toxic effects and high stability to heat treatment, the presence of mycotoxins in the food chain is potentially hazardous to the health of both humans and animals **[4]**.

Although the formation of mycotoxins in nature is considered a global problem. However, in certain geographical areas of the world, some mycotoxins are produced readily than others **[1, 5, 6]**.

Some mycotoxins are beneficial to man; they have been used as food, producing a mycoprotein called quorn derived from *Fusarium venenatum*. Quorn food is available in Europe and is eaten by millions of people. Also *Aspergillus Flavus* which produces aflatoxins are used in making food products such as soysauce. Some cultures are encouraged to grow on certain feeds in order to give the desired taste **[7]**.

Organisms like penicillium are used in the production of antibiotics under proper conditions. Mycotoxins can also be used as bioterriosm where aflatoxin and *Aspergillus parasiticus* are cultured and extracted to produce concentrated toxins and used to fill warheads **[8]**. Despite the advantages of some mycotoxins, several deleterious effects have been documented. They are known to be hepatotoxic, carcinogenic, mutagenic, immunosupressive, nephrotoxic, dermatotoxic, neurotoxic, teratogenic and immunotoxic **[6]**. Some mycotoxin producing organisms like *Aspergillus, Fusarium* and

Penicillum sp cause diseases including; systemic mycosis, cutaneous mycosis, subcutaneous mycosis, ear and eye infections. Since its discovery in numerous feedstuffs, aflatoxin, has caused much concern among consumers and producers alike. This toxin poses a serious economic threat to farmers **[9]**. Toxic and especially carcinogenic effects of aflatoxins have been reported in several different animals, aflatoxins cause liver damage, decreased milk and egg production, recurrent infection as a result of immunity suppression, in addition to embryo toxicity **[3]**.

Mycotoxins specifically aflatoxins have significant economic and commercial impact in that the safety, productivity and nutritive values of infected goods are affected **[6]**. This work therefore seek to investigate the prevalence of aflatoxins in some locally prepared food condiments with a view to creating in the society, food safety consciousness and the need for GMP and application of HACCP practices in preparation of food and food products.

MATERIALS AND METHODS

Sample Collection

A total of 32 samples comprising of four specimens each of *Citrullus vulgaris* (mellon), *Ricinus communis* (ogiri) *Brachystegia eurycoma* (Achi), *Origanum syriacum* (Offor), *Pleurotus tuber regium* (Osu), *Piper guineense* (Uziza), *Xylopia aethiopica* (Uda) and *Monodora mystrica* (Ehuru) were purchased from four (4) different locations in Owerri (Ihiagwa, Eziobodo, Nekede and Owerri metropolis). The samples were collected aseptically into sterile specimen containers and were adequately labeled and transported to the laboratory for analysis within one hour.

Analysis of Specimen

Ten gram (10g) samples were serially diluted 10^{-1} to 10^{-6}, the spread plate technique was adopted for culturing 0.2ml of sample homogenates onto PDA plus penicillin and streptomycin for fungal count and isolation. Incubation was at 28 ± 2°C for 3-5 days. Pure culture of isolates was obtained by repeated subculture on PDA. Fungal isolates were identified by macroscopic cultural characteristics and microscopy with reference to standard keys and atlas following the descriptions of **[10, 11]**.

Aflatoxin Assay

The RIDA QUICK Aflatoxin kit (R-Biopharm, UK) was used. It is an immunochromatographic assay based on antigen- antibody reaction and on the principle that a specific antibody against aflatoxin recognizes the aflatoxin molecules in the samples. The results are read visually

by observing the development of coloured band. The control band (control line) is not influenced by aflatoxin in the sample and should be present in all cases in order to prove that the test strip is valid. The test band (test line) is not visible in the absence of aflatoxin, it is only visible when aflatoxin is present in the sample.

Procedure

Ten gram (10g) of ground samples was weighed into suitable containers; 20ml of 70% methanol was added to the ground samples in the containers, capped and shaken vigorously for 3-5 minutes in shaker (Jenway, UK). The solution was allowed to sediment, and then filtered. Aliquot 50µ1 of the clear supernatant was added to 100µ1 of the temperate mobile solvent which was brought out of fridge and allowed to assume room temperature of 26±2°C. The solution was mixed and 100µ1 of it was applied on the application area of the test strip. The result was read after 4 – 16 minutes and interpreted based on the manufacturer's standard which stipulates: Negative result- The sample is free of aflatoxin or less than 4µg/kg(ppb) detection limit, if only the control band/line is clearly visible. Positive result- The sample is contaminated with aflatoxins if the control band/line is visible and the test band is also visible (20ppb after 4 minutes; 10ppb after 8 minutes; 4ppb after 16 minutes).

RESULTS

The result of the fungal culture for the eight different samples analyzed is shown in **Tables 1 and 2**. It reveals that all the samples except however; *Monodora mystrica* had high fungal contaminants.

Table 1 shows a high fungal count in most of the samples. Counts ranges from 1.2 $x10^4$ in *Pleurotus tuber regium* to 9.3 x 10^8 in *Origanum syriacum* and 8.2 x 10^9 in *Brachystegia eurycoma. Monodora mystrica* had few fungal growths within the incubation period. **Table 2** reveals the different fungal contaminants, Aspergillus and Penicillium spp are the most prevalent. *Ricinus communis* and *Piper guineense* had more mixed mycoflora compared to other samples. **Table 2** also show the aflatoxin assay, it reveals that two samples of *Brachystegia eurycoma* (Achi) and a sample of *Origanum syriacum* (Offor) had aflatoxin contamination of ≥20ppb (positive result in 4 minutes).

Table 1: Mean Fungal Count cfu/g of Samples

S. No.	SAMPLES	MEAN FUNGAL COUNT
1.	*Brachystegia eurycoma* (Achi)	8.2×10^9
2.	*Citrullus vulgaris* (mellon)	1.8×10^7
3.	*Monodora mystrica* (Ehuru)	< 10
4.	*Origanum syriacum* (Offor)	9.3×10^8
5.	*Piper guineense* (Uziza)	5.4×10^6
6.	*Pleurotus tuber regium* (Osu)	1.2×10^4
7.	*Ricinus communis* (ogiri)	8.8×10^6
8.	*Xylopia aethiopica* (Uda)	3.5×10^6

Table 2: Mycoflora and Aflatoxin Assay of Samples

S. No.	SAMPLES	MICROORGANISMS	AFLATOXIN
1.	*Brachystegia eurycoma* (Achi)	*Aspergillus favus, Aspergillus niger, Penicillium spp*	++
2.	*Citrullus vulgaris* (mellon)	*Fusarium spp, Penicillium spp, Mucor spp, Aspergillus fumigatus*	–
3.	*Monodora mystrica* (Ehuru)	*Rhizopus spp*	–
4.	*Origanum syriacum* (Offor)	*Penicillium spp, Aspergillus flavus, Rhizopus spp,*	+
5.	*Piper guineense* (Uziza)	*Aspergillus parasiticus, penicillium spp, Geotricum, Penicillium caseicolum, Aspergillus spp, Fusarium spp, Saccharomyces spp*	–
6.	*Pleurotus tuber regium* (Osu)	*Aspergillus spp, Candida spp, Mucor spp*	–
7.	*Ricinus communis* (ogiri)	*Penicillium spp, Aspergillus niger, Mucor spp, Aspergillus spp, Rhizopus spp*	–
8.	*Xylopia aethiopica* (Uda)	*Penicillium caseicolum, Rhizopus spp, Aspergillus spp, Penicillium spp*	–

DISCUSSION

The presence of fungi in all the samples could be attributed to the normal flora of the plants. Normal microbial flora has been reported to withstand processing procedures and conditions and be found in final products **[12, 13]**. Fungi are common environmental contaminants and are known to produce spores that survive unfavorable environmental conditions; this could explain their presence in the food condiments.

The high fungal counts of $\geq 10^6$ recorded for the samples are above standard specification **[14]**, the products are therefore not recommended for consumption without further treatments. The levels of contamination could be associated to contamination from the environment, the food vendors and personnel involved in the

production process **[15, 16, 17]**. It has been reported that the microbial contamination of a products is dependent on the environment it passed through and to the sanitary quality of the cultivation water, harvesting, transportation, storage, and processing of the produce **[12, 13, 18, 19**]. Ogiri and mellon condiments are fermented products often produced by mixed microbial flora due to poor local technology, contamination of these products by fungal species have been reported **[20, 21, 22]**. Uziza and Uda are spices mostly transported from the northern part of the country. Extensive handling during transportation could have contributed to the high contamination rate. The hardy nature of fungal spores could have supported the fungi in these spices that are known to have low water activity and have been reported to have some antimicrobial properties **[23, 24]**. Achi and offor are normally produced by boiling and grinding to powder the seeds of *Brachystegia eurycoma* and *Origanum syriacum.* The handling and grinding processes could be the sources of contamination, more so, the powdered seeds are not normally dried after processing to reduce water activity, this thus suggest that the products are of high water activity that could encourage the survival and proliferation of fungal contaminants and support aflatoxins formation. Achi and Offor are equally not fermented further suggesting that the medium have conducive condition that encouraged fungi proliferation and aflatoxins production.

Aflatoxins was not detected in Ogiri, Mellon, Uda and Uziza despite the very high fungal counts, this could be explained by lack of conducive condition for aflatoxin production. Ogiri and Mellon are fermented products, while Uda and Uziza are spices of low water activity and have been reported to contain antimicrobial substances. Aflatoxins have been reported to be produced only under conducive environmental condition of high water activity **[25]**. The presence of high fungal count above acceptable standard calls for concern and the detection of aflatoxigenic fungi and aflatoxin $\geq$ 20ppb is a cause for alarm. Education of producers of local food condiments, food vendors and consumers on the dangers of poor food storage and the need to adopt GMP and apply HACCP is advanced.

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